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ECOPHYSIOLOGICAL RESPONSES OF TWO TROPICAL URBAN SHRUB PLANTS TO ELEVATED CARBON DIOXIDE AND WATER STRESS

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ABSTRACT

Elevated atmospheric carbon dioxide causes changes in most plant species in terms of physiological and morphological properties. In this study, we elucidate the effects of elevated CO_2 and water regimes on two species of shrubs which were *Melastoma malabathricum* and *Clidemia hirta.* The factor of water stress was also included whereby for each CO_2 treatment, the amount of water was given once or twice daily. The treatment of elevated CO_2 was at 800 ppm, where the plants were exposed for two hours daily and ambient CO_2 at 400 ppm as the control. The growth of plants was monitored through their height, leaf area and biomass that were recorded fortnightly for six months. The physiological changes were determined via stomatal conductance, photosynthetic rate and transpiration rate. The results revealed that each species responded differently to the CO_2 treatments. The growth parameters of height and leaf area index showed positive responses on elevated CO_2 and twice watering for both species. As for biomass, *M. malabathricum* exhibited higher below ground biomass compared to above ground biomass in the elevated CO_2. In contrast, *C. hirta* showed the opposite trend. The results of the three physiological parameters demonstrated a bell shape trend for both treatments with the highest rate at the fifth month.

Key words: elevated CO_2, shrubs, photosynthesis, growth, tropical plant

INTRODUCTION

Climate change is principally driven by the burning of fossil fuels and greenhouse gas emissions from deforestation, degradation and land use change. As a result of the latter, the earth's natural capital is continuing to be badly degraded and thus affected water availability, soil stability, air quality, and forests ecosystem balance (International Sustainability Unit, 2015). The impacts of climate change has been considered as national security issue and may have many other indirect negative effects for livelihood and economy (Barnett, 2001). Enormous research efforts have been undertaken to understand how plants and ecosystems, both natural and managed, respond to rising CO_2 (Ainsworth & Long, 2005) and water stress (Corlett, 2016). For example, primary effects of rising CO_2 have been well documented and include reduction in stomatal conductance and transpiration, improved water-use efficiency, higher rates of photosynthesis and increased light-use efficiency of plants (Drake *et al.*, 1997). Whereas, plants under water stress were reported contribute an imbalance in biomass partition and also the productivity of the plants (Bastin *et al.*, 2015; Fauset *et al.*, 2015).

Effects of climate change and increased of carbon dioxide on the growth of individual plant species have been conducted on various kinds of plants. Most of the studies focused on crop plants such as *Ribes nigrum* and *Hordeum vulgare* L. (barley) (Johnson *et al.*, 2011; Robredo & Usue, 2007; Rogers *et al.*, 1996; Shimono *et al.,* 2013; Wang *et al.*, 2015), horticultural plants (Xu *et al.*, 2014), fruit trees (Schaffer *et al.*, 1999) and understory herbs native to Europe (Anderson & Cipollini, 2013). Most plants reacted positively to the increase in carbon dioxide (Niell, 2003) and also to other factors such as the content of nitrogen (Geisinger *et al.*, 1998), water regime, temperature and others. However, the effects of elevated CO_2 may vary across season (Anderson & Cipollini, 2013) and species itself. Rogers *et al* (1996) reported that, exposure of plant canopies to high concentration of

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carbon dioxide often stimulates the growth of both shoots and roots of the plants but there may be differences in allocation between root and shoot, at least for certain conditions. However, little did we know about the study involving tropical shrubs.

Shrubs are important for moderating a range of ecosystem services because they influence micro-climate, reduce erosion and nutrient loss, increase water holding capacity, maintain soil structure and stability and provide habitat for a range of taxa (Maestre *et al.*, 2009). It has been proposed that disturbances ranging from climate change to grazing to fire suppression can generate heterogeneity in soil resources and thus create opportunities for shrub colonization (Schlesinger *et al.*, 1990).

Melastoma malabathricum L. and *Clidemia hirta* L. from *Melastomataceae* family are amongst the widely distributed shrubs in tropical open areas or urban forests. *M. malabathricum* can be found abundantly in Malaysia. Additionally, this plant has been reported to be found growing wild in the Indian Ocean Islands, throughout South and South-East Asia, China, Taiwan, Australia and the South Pacific Ocean (Wong, 2008). This species is a common herbal plant used in folk treatment to treat inflamed wounds (Omar, 2012), while leaf, root and also flower crude from this species had been used to treat toothache, diarrhea, scar prevention, wounds, post-partum recovery and others (Joffry *et al.*, 2012). As for *C. hirta,* its natural range is extremely wide, extending throughout most of the humid tropical portions of Central and South America from Southern Mexico to Northern Argentina and the islands of the West Indies. It is characteristically found in moist, shaded localities on the edges of clearings and stream-banks, in ditches, and along paths and roadways (Wester & Wood, 1977). Medicinally, *C. hirta* is the second plant preferred after *Anacardium occidentale* L. to treat leishmanial ulcers (Franca *et al.*, 1996).

In this study, we examined the effects of elevated CO_2 and water regime on two tropical shrub species of *M. malabathricum* and *C. hirta* in terms of growth and physiology. The two shrub species were selected because they grow wild and commonly found in urban forest areas. Since both selected plant species are widely dispersed at urban forested areas, we hypothesized that elevated carbon dioxide concentration in their surroundings will positively change the plant growth morphologically and physiologically and will in turn affect their abundance in the urban forest areas. Meanwhile, the different water regime is expected to reveal the physiological mechanisms of the two tropical shrub plants adapting to water stress.

MATERIALS AND METHODS

Plant materials

Seedlings of *M. malabathricum* were obtained from seeds that were collected from mature plants which grew wild along the roadsides of UKM Bangi campus (2.9192° N, 101.7691° E). Meanwhile, for *C. hirta*, stem cuttings of the mature plants that grew at similar location were used instead, after several attempts made to obtain seedlings from its seeds were unsuccessful. The seeds and the stem cuttings were then planted in nursery trays filled with topsoil and watered daily until the seedlings emerged which, were around seven to ten days after planted. The growing medium used was topsoil of podzolic type mixed with organic fertilizer with a ratio of 4:1. After the saplings reached the age of 2 weeks old, each sapling were planted in a 8 x 6 inches polybag with NPK fertilizer at the ratio of 15:15:15 at a low dose of 5 g/polybag/month.

Experimental design

The plants were then divided into four groups of treatments. Two factors taken into account were the concentration of carbon dioxide and water regime. Each factor had 10 replications of saplings. The concentration of carbon dioxide for ambient as the control was at 400±50 ppm and elevated CO_2 was at 800±50 ppm. For the ambient CO_2 treatment, the saplings were grown in a shade house. Saplings exposed to elevated CO_2 were in an open roof greenhouse. The elevated CO_2 was injected for two hours from 0900 am until 1100 am, daily. The roof is covered with a double layer of UV protected polycarbonate material. The side wall is covered with mesh netting and insect-proofed. A centralized control panel is installed to control all of the systems and monitor relative humidity, temperature and carbon dioxide concentration in the greenhouse. For each treatment of carbon dioxide, saplings were divided into two water regimes where they were either watered once or twice daily. The amount of water given each time was approximately 500 mL.

Growth measurements

Growth parameters of each sapling were measured fortnightly for six months from April 2015 until September 2015. The growth parameters presented here are height, leaf area index and biomass as these growth measurements reflected the percentage of growth for the plants in the experiment by analyzing the trends of its increment. However, the three parameters are inter-related. For example, the increment in height and leaf area will thus affect its biomass composition. As for the leaf area, all parts

of the leaves were laid on a paper then digital images were taken with a ruler as the reference scale. The leaf area was next calculated using the software of ImageJ. Measurements for stem height were made from the base of the stem up to its apex. At the end of the treatment, all of the plants were harvested and both the above ground and below ground biomass were obtained. The fresh weight of biomass was measured using a digital scale up to two decimal points. Dry weight measurements were conducted after the plants were dried in the oven at a temperature of 65°C for seven to ten days.

Physiological parameters

Physiological changes of plants were monitored using the photosynthetic rate, transpiration rate and stomatal conductance. These parameters were chosen because photosynthetic rate do affects the growth of the plant (Kirschbaum, 2011) meanwhile, stomatal conductance is closely related to the rate of photosynthesis (Farquhar & Sharkey, 1982) so as the transpiration rate. For this physiological data, we used portable infrared gas analyser (Li-6400, Li-Cor, Lincoln, NE, USA).

Statistical analysis

Differences between means of all measured parameters were tested using the General Linear Model (GLM) in Minitab version 17 at the significance level of 5% ($p< 0.05$).

RESULTS AND DISCUSSION

Effect of Elevated CO_2 and Water Regime on Plant Height

Plants of *M. malabathricum* in ambient and elevated carbon dioxide showed a significant different ($p< 0.05$) where the height of *M. malabathricum* was higher at the elevated than the one in ambient CO_2 (Figure 1). However, the water treatment did not show a significant different of plant heights ($p=0.34$). The stem height of *M. malabathricum* showed the highest increment for plants grown in elevated CO_2 with 240% and 193% of height increment for once and twice watered, respectively. Meanwhile, in the ambient CO_2 the height increment was 106% and 107% for once and twice watered, respectively. Our results clearly indicated that *M. malabathricum* stem growth reacted positively with the increase of CO_2 concentration.

Contrary to the *M. malabathricum, C. hirta* on the other hand, showed the highest increase in percentage of mean stem height were for plants grown in ambient and once watered which was 232% of height increment followed by elevated CO_2 and twice watered with percentage of increase at 174%. Meanwhile, the treatment of elevated CO_2 and once watered showed only 146% of *C. hirta* stem height increment. In short, the two selected shrub plant responded differently to the elevated

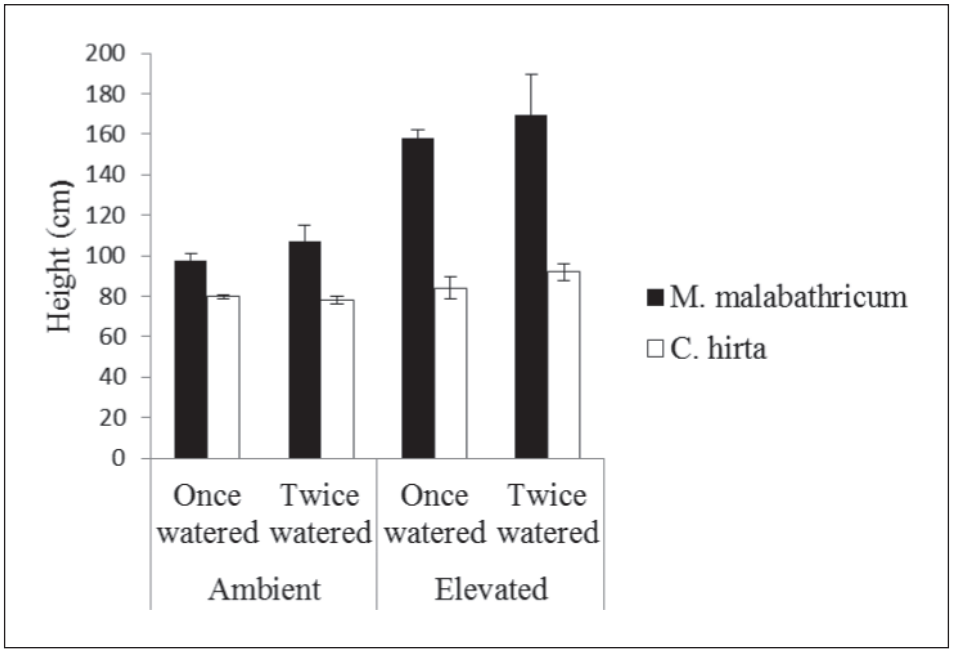

Fig. 1. Effect of elevated CO_2 concentration and water regime on the mean (±SE) height of *M. malabathricum* and *C. hirta* ($p< 0.05$).

CO_2. Individual plant organs have often been observed to enlarge proportionally with increasing CO_2 such as increasing stem and roots partitioning (Allen *et al*., 1990) and also the expanse and thickness of leaves increased (Thomas & Harvey, 1983).

For the six months of the elevated CO_2 treatment, an analysis on the plant height relative growth rates (RGR) of *M. malabathricum* exhibited that at elevated CO_2, an increase of RGR was evident at the earlier treatment but after the second month, a continuous decrease of RGR was observed (Figure 2). The *M. malabathricum* plants in ambient CO_2 also displayed similar trend except that the RGR was higher at the elevated CO_2. Although the elevated CO_2 treatment increased the RGR of *M. malabathricum* stem height and consequently the mean RGR, past studies showed that the enhancement might be reduced if the treatment is prolonged (Ceulemans & Mousseau, 1994). Meanwhile, *C. hirta* showed a high RGR of stem height for the first month but, sharp decreases of RGR for the remaining months. Compared to *M. malabathricum*, the *C. hirta* displayed insignificant differences ($p > 0.05$) between treatments of water regime and CO_2 concentration.

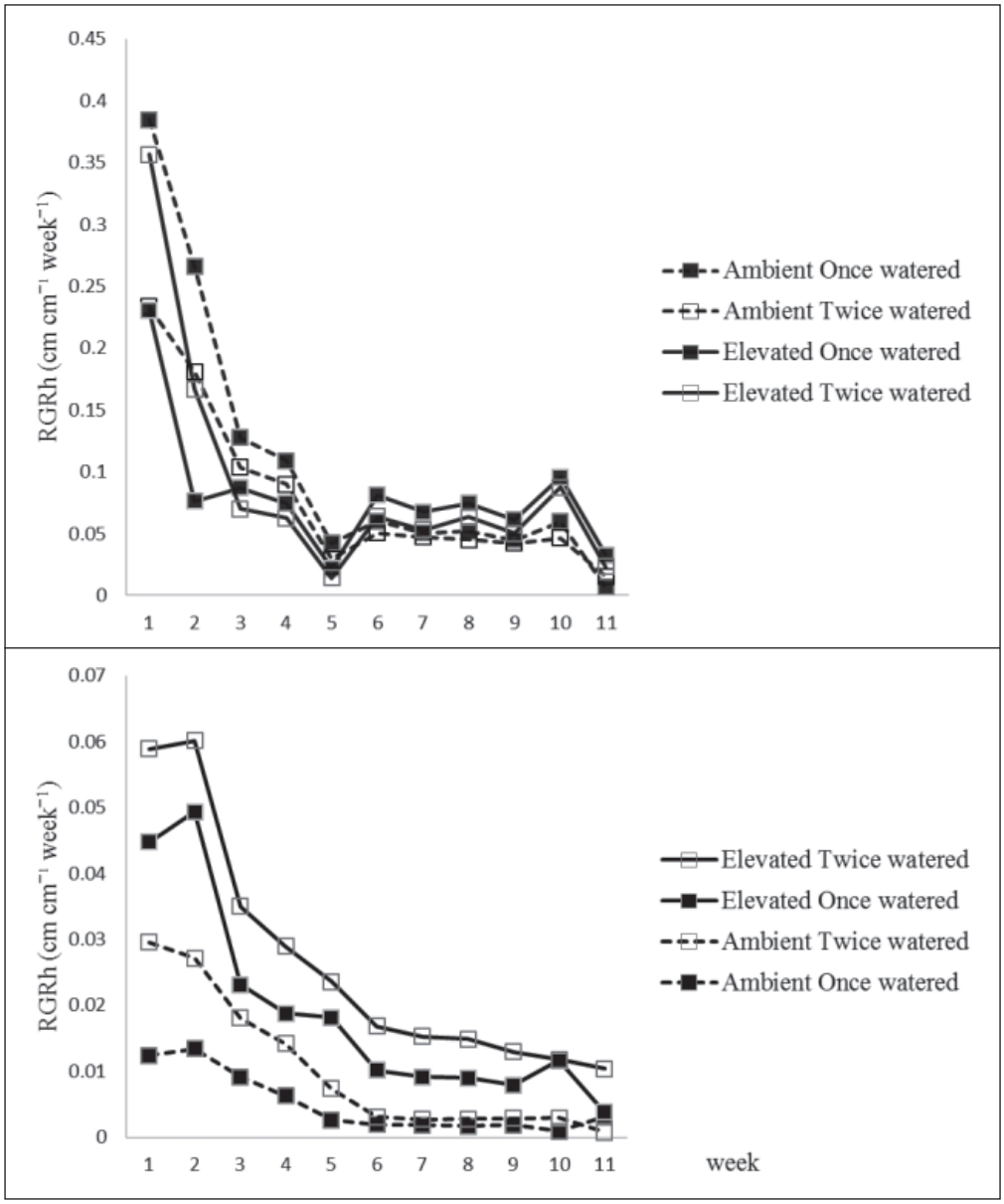

Fig. 2. Time course of relative growth rate for the height of *Clidemia hirta* (above) and *Melastoma malabathricum* (below) seedlings for eleven weeks at ambient (400±50 ppm) and elevated CO_2 (800±50 ppm) and two water regime.

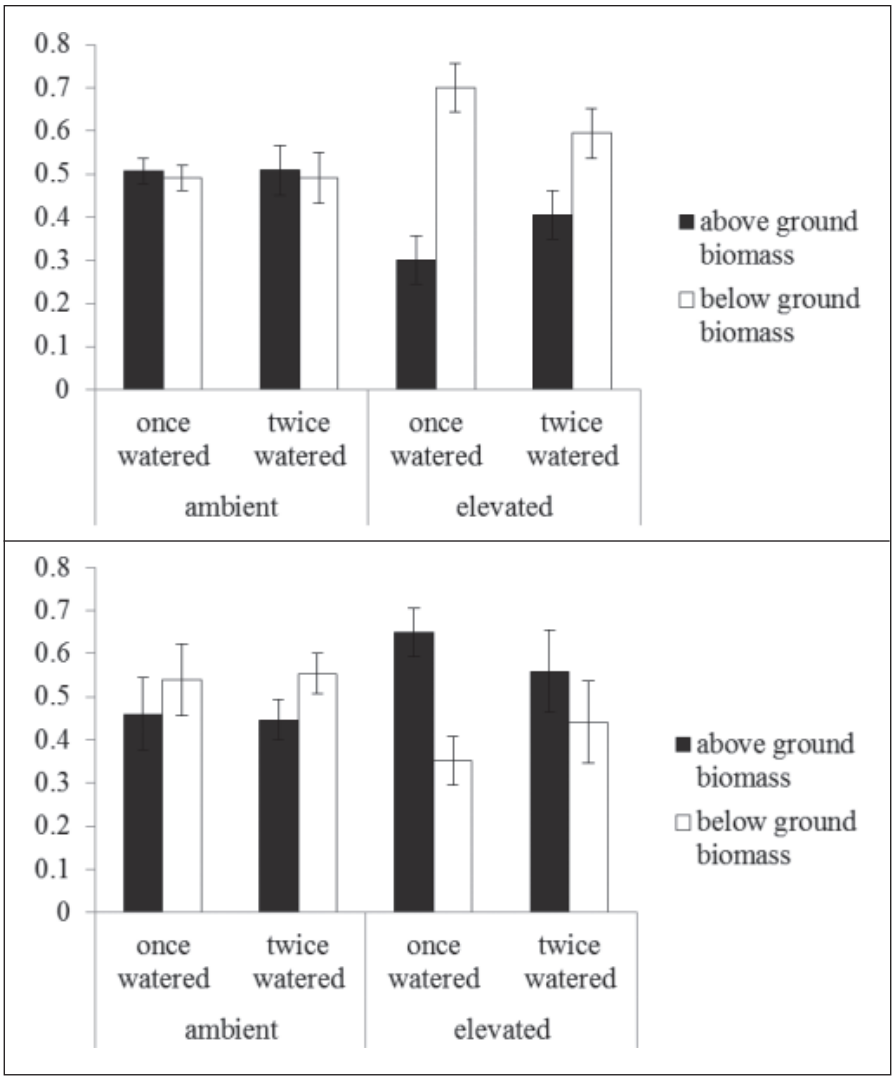

Fig. 3. Effect of elevated CO_2 concentration and water regime on the mean (±SE) biomass partitioning of *M. malabathricum* (above) and *C. hirta* (below) ($p < 0.05$).

Effect of Elevated CO_2 and Water Regime on Biomass Allocation

The *M. malabathricum* responded to elevated CO_2 by increasing below ground biomass. On the contrary, *C. hirta* had higher aboveground biomass in elevated rather than in ambient CO_2 (Figure 3). The differences between plant species may due to the partitioning of photosynthetic which may be influenced by environmental stimuli as stated by Rogers *et al* (1996) and it differs for different species. Our results agrees with Farrar and Gunn (1996) that there are no reason to assume that shoots will increase or decrease more than roots. In addition, Geisinger *et al* (1995) demonstrated that root volumes for below ground growth largely mirrored that of the shoot, or at least do not deviate sufficiently to substantially modify the growth pattern. This concludes that different species offers different results in its biomass allocation.

Water regime did not significantly affect plant biomass allocation at both CO_2 concentrations for both species ($p > 0.05$). However, plants in the elevated CO_2 treatment had higher differences when compared between once and twice watered whereas, in ambient CO_2 not much differences between plants of once and twice watered. This may due to the interaction of water regime and CO_2 treatment ($p < 0.05$). Plants exposed to higher CO_2 may have a better increase in biomass as the amount of water given was sufficient for plant growth.

Effect of Elevated CO_2 and Water Regime on Leaf Area Index

Elevated CO_2 significantly increased the leaf area index of both species. An interaction of water regime and CO_2 gave significant ($p < 0.05$) effect on the leaf area index of both species except for twice watered *C. hirta*. For *M. malabathricum*, the

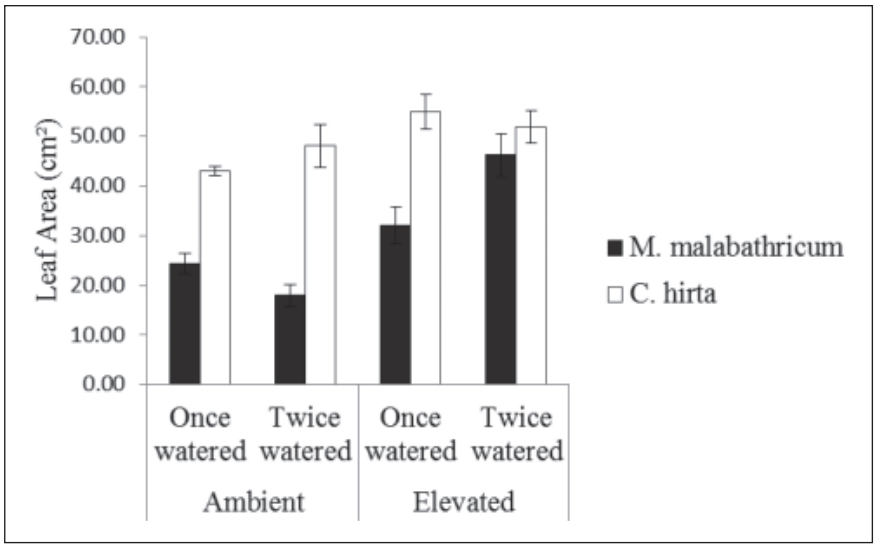

Fig. 4. Effect of elevated CO_2 concentration and water regime on the mean (±SE) leaf area index of *M. malabathricum* and *C. hirta* ($p< 0.05$).

highest mean leaf area was 46.24 cm^2 in the elevated CO_2 and twice watered followed by elevated CO_2 and once watered of 32.06 cm^2. Plants of *M. malabathricum* in ambient CO_2 had smaller size of leaf area with the mean value of 24.35 cm^2 and 17.94 cm^2 for once and twice watered, respectively. Our results suggested that the effect of water stress on *M. malabathricum* leaf area was more pronounced when grown in elevated than ambient CO_2.

Similar effect of interaction between CO_2 and water regime were shown by the plants of *C. hirta* except that its leaf area was bigger compared to the *M. malabathricum* (Figure 4). *C. hirta* plants in the elevated CO_2 and once watered exhibited the highest mean leaf area with the value of 55.0 cm^2 followed by plants in elevated CO_2 and twice watered of 51.8 cm^2 ($p< 0.05$). Contrary to the elevated CO_2, the *C. hirta* plants had larger size in ambient CO_2 and twice watered compared to plants in ambient and once watered with the value of 48.2 cm^2 and 43.00 cm^2, respectively ($p< 0.05$), indicating the effect of water stress on *C. hirta* leaf area was more pronounced in ambient CO_2.

Our results is in line with Sasek and Strain (1989) which revealed that changes in plant water status have been identified as mechanism for increased leaf area in elevated CO_2. Previous study on meta-analysis of soybean (*Glycine max*) showed that when this plants was exposed to elevated CO_2, their average leaf area index (LAI) increased by 18% relative to ambient CO_2 (Ainsworth *et al.*, 2002). However, the leaf area in elevated CO_2 has been found to vary both among and within plant species itself (Taylor *et al*., 1994; Masle, 2000).

Effect of Elevated CO_2 and Water Regime on Photosynthesis Rate (P_s)

Six month's exposure of ambient and elevated CO_2 resulted in a variation of bell curve-shaped $\mathbf{P_s}$ of *M. malabathricum* for both once and twice watered. A higher rate for the first two months than the remaining months in the ambient CO_2 was observed for *M. malabathricum* plants (Figure 5). The highest value of $\mathbf{P_s}$ was the *M. malabathricum* grown in ambient and once watered at the second month with the value of 16.3 μmol CO_2 m^{-2} s^{-1} followed by the $\mathbf{P_s}$ of the first month with the value of 16.1 μmol CO_2 m^{-2} s^{-1}. The highest $\mathbf{P_s}$ in elevated CO_2 was the one with twice watered at 13.7 μmol CO_2 m^{-2} s^{-1} which had the same $\mathbf{P_s}$ at ambient and twice watered. A lower $\mathbf{P_s}$ in elevated CO_2 showed that *M. malabathricum* able to adapt and take an advantage on the increasing CO_2 in urban areas.

As for *C. hirta*, when the plant was exposed to elevated CO_2, over time, similar variation of bell-shaped $\mathbf{P_s}$ was observed with an exception of exponential $\mathbf{P_s}$ in elevated CO_2 and twice watered. The highest $\mathbf{P_s}$ of *C. hirta* were the plants in elevated CO_2 and once watered at the value of 11.1 μmol CO_2 m^{-2} s^{-1} at the third month followed by $\mathbf{P_s}$ of *C. hirta* in ambient and once watered with the value of 10.2 μmol CO_2 m^{-2} s^{-1} at the fourth month.

In short, the two species' $\mathbf{P_s}$ react differently to elevated CO_2 ($p< 0.05$). Photosynthetic rate for *M. malabathricum* was lower in elevated than in ambient CO_2 whereas for *C. hirta* the $\mathbf{P_s}$ was higher in elevated than in ambient CO_2. Meanwhile, water regimes did not significantly ($p= 0.10$) affect the $\mathbf{P_s}$ of both species at both CO_2. Our results correspond

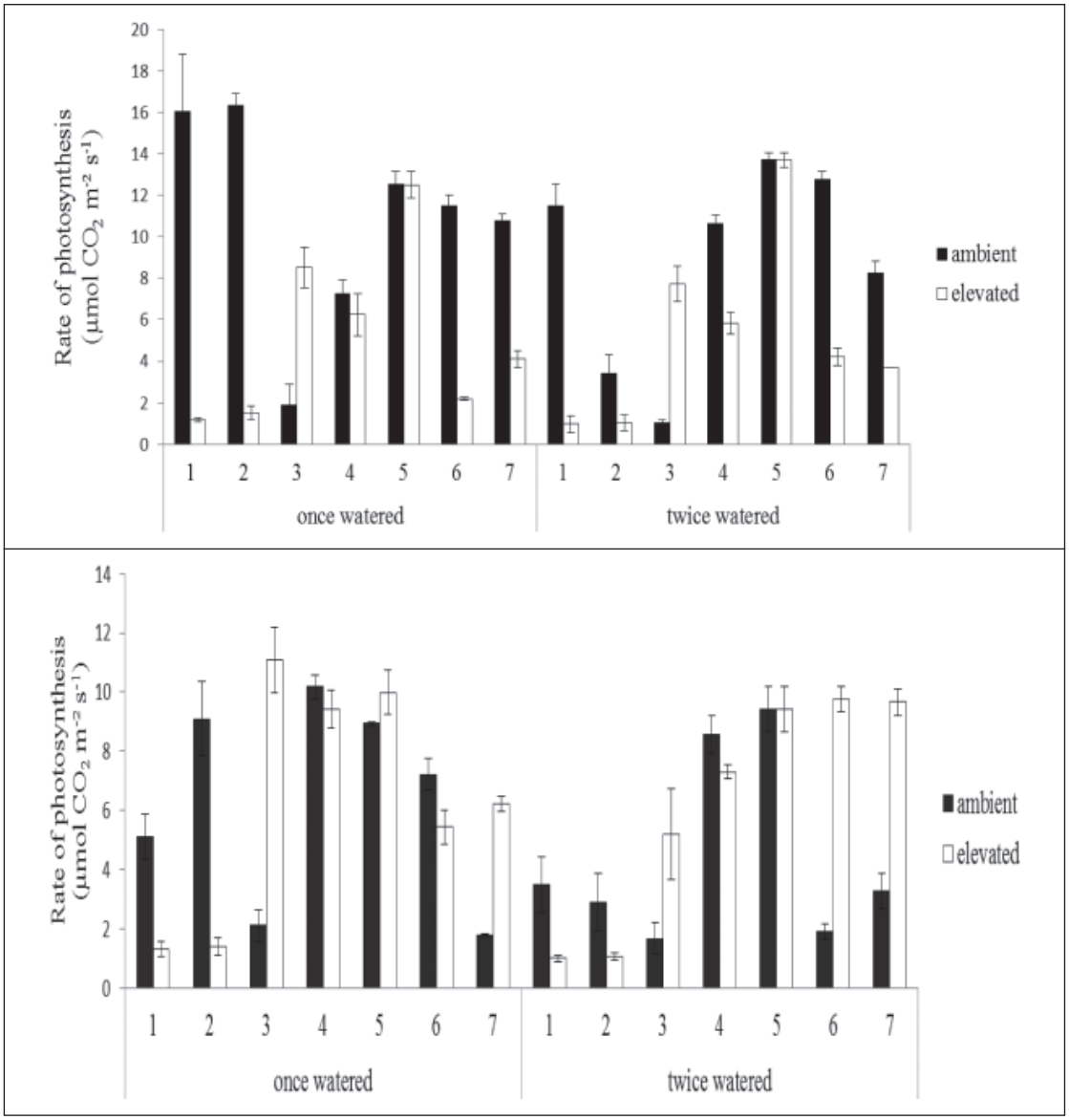

Fig. 5. Effect of elevated CO_2 concentration and water regime on the mean (±SE) photosynthesis rate of *Melastoma malabathricum* (above) and *Clidemia hirta* (below) ($p < 0.05$).

to a study by Nagy *et al* (2000) whereby CO_2 treatment do affects the values of $\mathbf{P_s}$ but not the pattern. As CO_2 increased over time, the $\mathbf{P_s}$ would initially increase and started to decrease after a certain period of plant growth. Our results also indicated that the interactions between species and CO_2 level do affect the rate of photosynthesis significantly rather than species-water regime and CO_2 level-water interactions.

Effect of Elevated CO_2 and Water Regime on Stomatal Conductance (g_s)

The $\mathbf{g_s}$ response of *M. malabathricum* during the six months CO_2 exposure was a bell curve trend for both ambient and elevated treatments. A lower rate of $\mathbf{g_s}$ was found for *M. malabathricum* plants grown in the elevated than ambient CO_2 ($p < 0.05$). The highest $\mathbf{g_s}$ of *M. malabathricum* was on the fifth month of twice watered plants in ambient and elevated with the same value of 0.6 mol H_2O m^{-2} s^{-1}. However, there was not much difference in both CO_2 treatments related to different water regime (Figure 6).

Correspondingly, for *C. hirta*, plants in elevated CO_2 also had a lower rate of $\mathbf{g_s}$ when compared to the one in the ambient CO_2. Our results also showed that a high $\mathbf{g_s}$ of *C. hirta* at the first two months in ambient CO_2 for both water regime treatments. The least $\mathbf{g_s}$ rate of *C. hirta* was in elevated CO_2 and twice watered. The highest $\mathbf{g_s}$ of *C. hirta* was the plants in ambient and twice watered with the value of 0.9 mol H_2O m^{-2} s^{-1} at the fifth month followed by the one in ambient and once watered at the second month with the value of 0.6 mol H_2O m^{-2} s^{-1}.

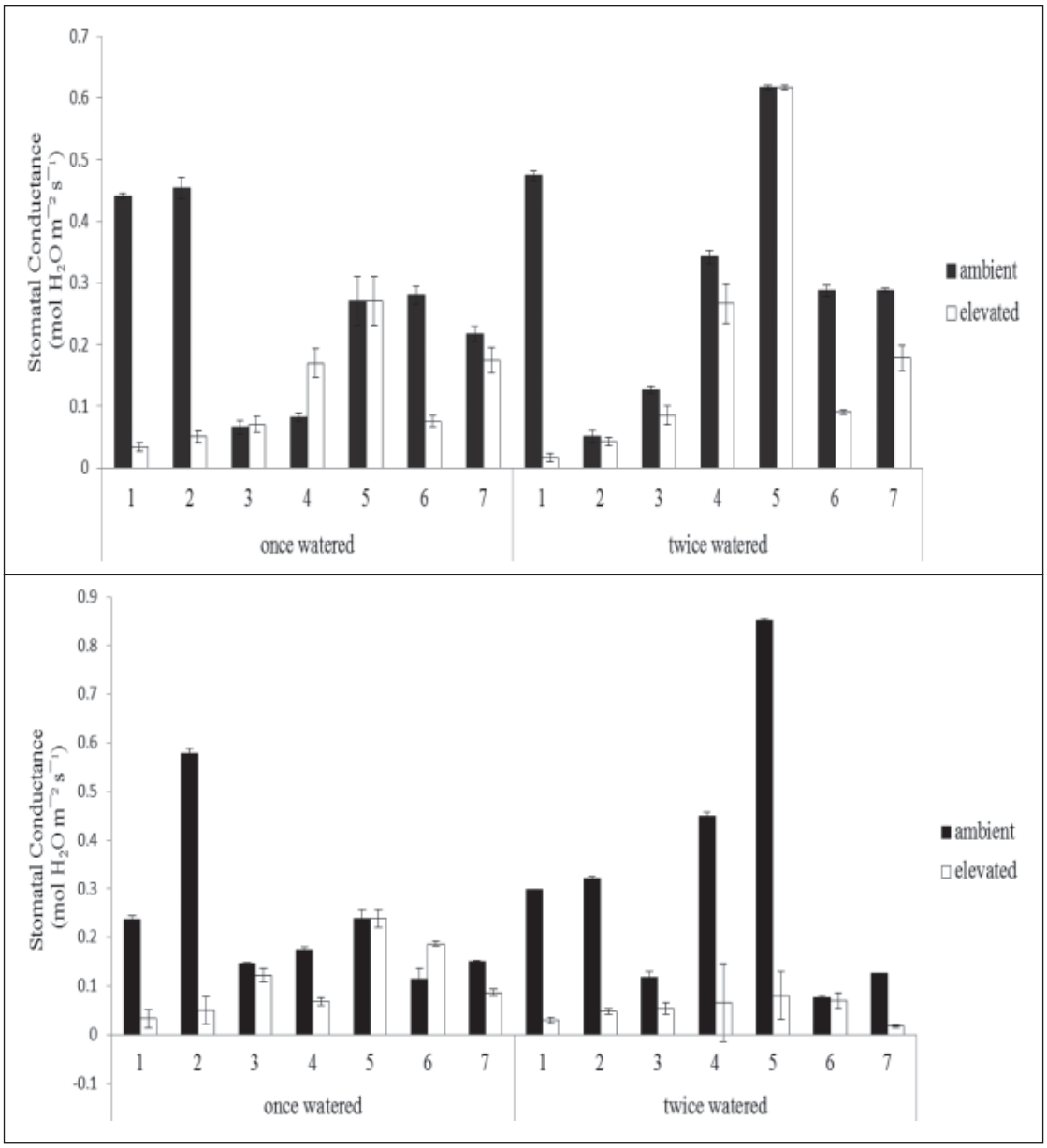

Fig. 6. Effect of elevated CO_2 concentration and water regime on the mean (±SE) stomatal conductance of *Melastoma malabathricum* (above) and *Clidemia hirta* (below) ($p< 0.05$).

Robredo and Usue (2007) reported that stomatal conductance decline with increasing CO_2 but the rate of reduction is higher under sufficient water rather than under water stress. The lower stomatal conductance in elevated CO_2 was also reported by Roberntz and Stockfors (1998) in their experiment on *Picea abies* (L.) Karst. They suspected that these two species are less affected by elevated CO_2 compared to the one that have higher stomatal conductance as stated by Morison (1985). A decrease in stomatal conductance in elevated CO_2 may due to a decrease in foliar nitrogen concentration in response to elevated CO_2 (Roberntz & Stockfors 1998). Moreover, Woodrow (1994) suggested that C_3 plants possess a genetically controlled feedback mechanism to smooth out imbalances within photosynthetic system caused by a rise in atmospheric CO_2 concentration.

Effect of Elevated CO_2 and Water Regime on Transpiration Rate (E)

Responses of this two plant species transpiration rate to differences in CO_2 and water regimes showed that a lower **E** was observed at the elevated than ambient CO_2 ($p< 0.05$). Meanwhile, water regime did not significantly affect the **E** of both species at both

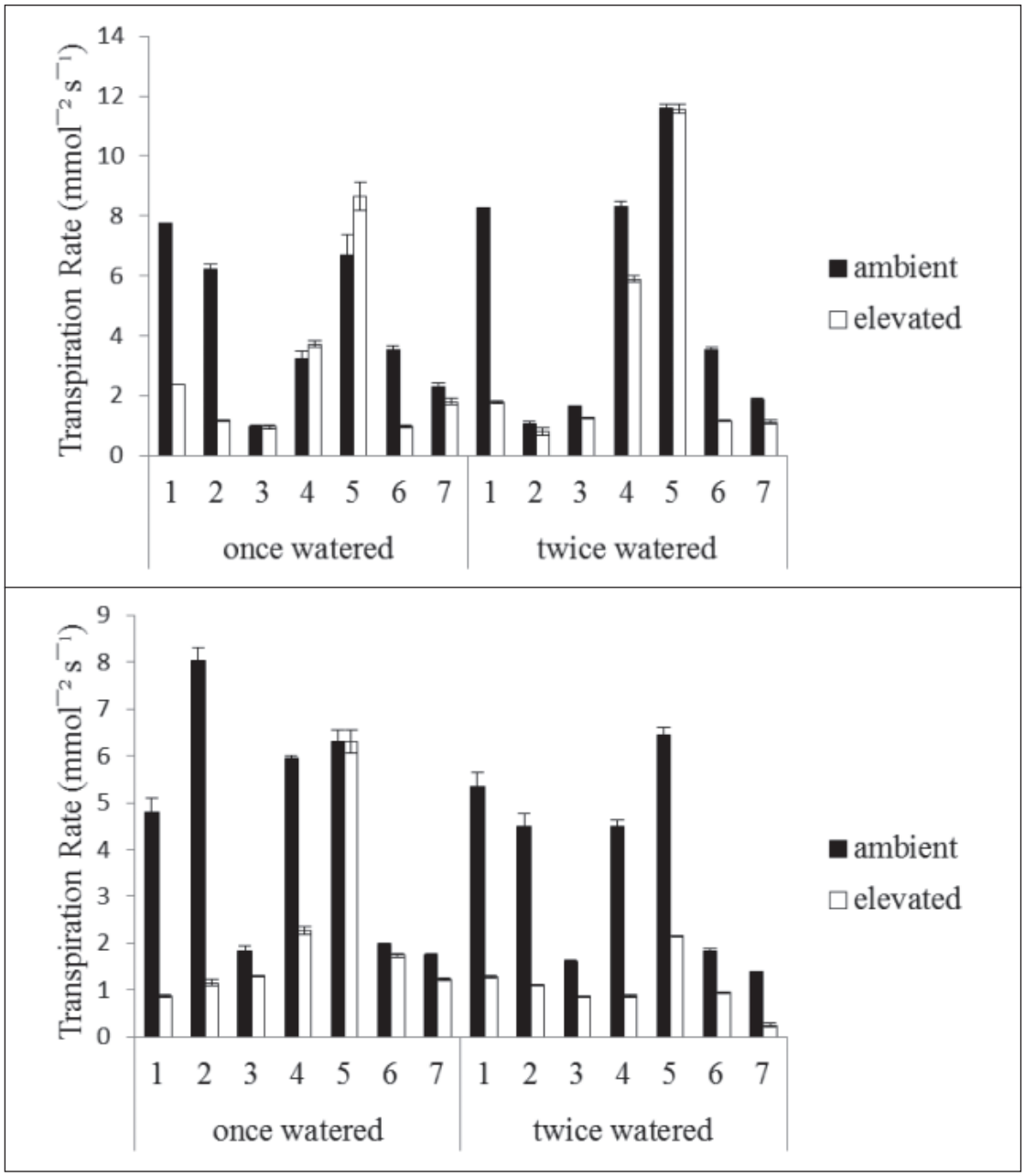

Fig. 7. Effect of elevated CO_2 concentration and water regime on the mean (±SE) transpiration rate of *M. malabathricum* (left) and *C. hirta* (right) ($p< 0.05$).

CO_2 ($p> 0.05$) (Figure 7). Our results differ than findings reported by Wang *et al* (2015), whom observed a higher rate of transpiration at elevated CO_2 on *Gynura bicolor*. The contrasting finding is possible as different types of plants react differently to elevated CO_2 and also water regime.

The highest value of **E** for *M. malabathricum* was observed in both elevated and ambient CO_2 and twice watered with the difference of **E** value of only 0.01 $mmol^{-2}s^{-1}$ at the fifth month. The highest **E** for *M. malabathricum* plants in elevated CO_2 was on the fifth month and once watered with the value of 6.7 $mmol^{-2}s^{-1}$. Meanwhile, *C. hirta* had the highest value of **E** at ambient and once watered in the second month of treatment with the value of 8.0 $mmol^{-2}s^{-1}$ followed by the one in ambient and twice watered with the value of 6.5 $mmol^{-2}s^{-1}$ at the fifth month.

A lower rate of transpiration for the plants in elevated CO_2 compared to the ambient CO_2 may due to the diffusive resistance of H_2O is higher compared to the diffusive resistance of CO_2 and directly demonstrated that the plants in elevated CO_2 allowed the gas to diffuse into the leaves while they prevent H_2O from being released in order to protect themselves from dehydration (Drake *et al.,* 1997). Thongbai *et al* (2010) also pointed out that

transpiration will decrease as the CO_2 concentration increased due to an increased in air circulation. This means that transpiration rate and rate of stomatal conductance do affect each other at high CO_2 concentrations.

CONCLUSIONS

Positive effect of elevated CO_2 was more prominent for *M. malabathricum* compared to *C. hirta*. Morphologically, the positive effect of elevated CO_2 was exhibited by significant increase of plant height, below ground biomass and leaf area index of *M. malabathricum*. Whereas, for *C. hirta*, the positive effect of elevated CO_2 was seen in smaller increment of above ground biomass (instead of below ground biomass) and leaf area index. Both shrub species was morphologically not affected by water stress possibly due to their adaptation to extreme environment in urban areas. However, an interaction between CO_2 and water regime had the most significant effect on the leaf area index of both species.

The physiological responses of the two shrub species indicated that they are able to take advantage on elevated CO_2 concentration in urban areas, particularly for *M. malabathricum* and to a lesser extent for *C. hirta*. The different strategy used by the two species to cope with increased CO_2 may lies on the different natural microhabitat of these two species. *M. malabathricum* is a light-demanding shrub whereas *C. hirta* thrived better under shady habitat. It is also possible that confounding effects of other environmental factors such as light intensity may limit the two shrub plants response to elevated carbon dioxide. Therefore, we suggest other important growth limiting factor of different species should be considered in future elevated CO_2 experiment.

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